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**Cowpea Flour, Whey Protein Fortification of Rice Starches: Effects
on Antioxidant and Starch Digestibility and Starch Pasting
Properties**

A Dissertation
submitted in partial fulfilment
of the requirements for the Degree of
Master of Science in Food Innovation

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by
Shijie Guo

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2020

Abstract of a Dissertation submitted in partial fulfilment of the
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by

Shijie Guo

Rice contains more starch, less protein and dietary fibre compared with other cereal. Cowpea is one of the important legumes with high nutrition content. It is rich in proteins, complex carbohydrates, dietary fibres, bioactive compounds, vitamins and minerals. Generally, rice flour has a high glycemic index (GI), while legume flour is considered as low GI food due to the high dietary fiber and slowly digestible starch content. Therefore, it is an excellent way to improve the nutrition of the rice starch product and manipulate the starch digestibility by incorporating protein and legume flour (such as cowpea flour) to rice flour. However, the fortification of protein and legume flour also might affect the pasting property of the blended flour due to the synergistic effect of protein, starch and dietary fiber. The objective of this study is to examine the effect of incorporating both legume flour (cowpea flour) and whey protein to rice flour on the antioxidant properties, pasting attributes and starch digestibility of the blended flour composed of different ratio of cowpea flour, whey protein concentrate (WPC) and rice flour. Five formulations were studied. There is a significant positive correlation between mean total phenolic content (TPC) of the samples and the proportion of cowpea flour incorporated ($P \leq 0.05$). Also, there is a significant positive correlation between TPC with ABTS radical scavenging capacity ($P \leq 0.05$) of the samples. According to the analysis of RVA results, the addition of cowpea flour and whey protein has a significant effect on the pasting properties of the blended flour. The peak, breakdown and final viscosity of samples decreased gradually with the increasing proportion of cowpea flour and whey protein concentrate. However, according to ANOVA analysis and Tukey's comparison test of RVA results, the peak viscosity of Formulation 1 to formulation 3 and cowpea flour, rice flour is significantly different ($P \leq 0.05$) while there is no significant difference between Formulation 4, 5 samples and cowpea flour in peak viscosity ($P > 0.05$). This means the peak viscosity increased significantly by the incorporation of cowpea flour and whey protein at a low level, while the influence on peak viscosity became not significant at high-level addition. Similarly, the breakdown values also did not significantly differ among Formulation 2-5

samples, which means a low concentration of cowpea flour 10% has a significant effect on breakdown viscosity($P \leq 0.05$), while the effect of higher-level incorporation was not significant($P > 0.05$). The final viscosity differed significantly among all samples ($P \leq 0.05$). Based on the in vitro starch digestion analysis, the incorporation of whey protein and cowpea flour affected the starch digestibility of samples. Overall, the amount of reducing sugar released of the samples decreased during in vitro starch digestion with the increased proportion of whey protein and cowpea flour in the formulations due to the decrease in starch and increasing of slowly digestible starch from cowpea flour, and the synergistic effect of protein, starch and dietary fiber. The effect of cowpea flour added in rice flour on the pasting property and starch digestibility needs to be further studied using a higher proportion of cowpea flour.

Keywords: cowpea flour, rice starch, starch digestibility, pasting property, whey protein

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Table of Contents

Abstract	ii
Acknowledgements	iv
Table of Contents	v
List of Tables	vi
List of Figures	vii
Chapter 1 Introduction	1
1.1 Objective	4
1.2 Hypotheses	4
Chapter 2 Material and Methods	5
2.1 Materials	5
2.2 Antioxidant property measurement	5
2.2.1 Preparation of sample extracts	5
2.2.2 Total Phenolics content (TPC)	5
2.2.3 Measurement of Radical Scavenging Capacity (ABTS)	5
2.3 Viscosity Measurement using Rapid Visco Analyzer (RVA)	6
2.4 In Vitro Starch Digestion	7
2.5 Statistical analysis	7
Chapter 3 Results and Discussion	8
3.1 Antioxidant property	8
3.1.1 Total Phenolics Content (TPC)	8
3.1.2 Measurement of ABTS Radical Scavenging Capacity	9
3.2 Viscosity Measurement by RVA	11
3.3 In Vitro Starch Digestion	14
Chapter 4 Conclusion	17
References	19

List of Tables

Table 2-1 Sample recipes	5
Table 2-2 The temperature and time profile for RVA analysis.....	6
Table 2-3 Raw cowpea flour, rice flour, and five formulation samples for RVA analysis. The rice flour was added with cowpea flour and whey protein concentrate (WPC) as five different formulations (F1-F5).....	7
Table 3-1 The content of total phenolics of cowpea flour, rice flour, and the five different ratio formulation samples.	8
Table 3-2 Results of Radical Scavenging Capacity (ABTS) of raw cowpea flour, rice flour, and five formulation samples.	9
Table 3-3 Results of RVA analysis of raw cowpea flour, rice flour, and five formulation samples.....	11

List of Figures

Figure 3-1 Regression analysis of the percentage of cowpea flour and TPC of samples ($P \leq 0.05$)	8
Figure 3-2 Regression analysis of ABTS Radical Scavenging Capacity and TPC of samples ($P \leq 0.05$)	9
Figure 3-3 The glucose released (mg glucose/g sample) during in vitro starch digestion of cowpea flour, rice flour, and five different formulations of cowpea flour, rice flour and whey protein at 0, 20, 60, 120 mins.	14
Figure 3-4 Values for area under the curve (AUC) of five different formulations of cowpea flour, rice flour and whey protein.....	14

Chapter 1

Introduction

Starch is a primary ingredient in many recipes of processed food because of both the nutrients and the capability to modify the texture of the food product (Villanueva, Ronda, Moschakis, Lazaridou, & Biliaderis, 2018). Rice is one of widely consumed cereal grain in the world. Rice flour has been used to produce many food products, such as pasta, noodle, cake, and extrusion products. However, rice contains more starch, less protein and dietary fibre compared with other cereal grain (Oñate Narciso & Brennan, 2018). Therefore, it is necessary to incorporate protein and dietary fibre to improve the nutrition of the rice starch product. Fortification of protein and dietary fibre to rice starch to produce food product have been widely studied recently.

Pulses (such as chickpea and cowpea) are not only a sustainable source of plant protein but also a good source of dietary fibre (Bessada, Barreira, & Oliveira, 2019). The protein proportion in legume flours is about 20% to 30% db. Dietary fibre contents are much higher in legume flour (10% db in pea and faba flours, 20%–40% db in chickpea, lentil and lupin flours) than wheat flour (about 2% db) (Monnet, Laleg, Michon, & Micard, 2019). Cowpea is an important legume with high nutrition content. It is rich in proteins, complex carbohydrates, dietary fibres, vitamins and minerals. The protein content (20.3 - 39.4 g/100 g) in cowpea seeds is much higher than that of cereals (3–7g/ 100 g). Also, cowpea proteins contain high-level essential amino acids, especially lysine, histidine and aromatic amino acids while rice proteins contain less lysine (Fabian & Ju, 2011). Therefore, cowpea can be complementary to the nutrition of cereal grains to produce a cereal-based product with better amino acids profile (Adjei-Fremah et al., 2019).

The effect of bioactive compounds in legumes on human health has been a hot topic (Adjei-Fremah et al., 2019). Pulse seeds pigmentation contains high-level phenolic compounds, including phenolic acids, tannins, and flavonoid (for example, anthocyanins) in the diet of human (Bessada et al., 2019), it is believed that phenolics are important antioxidant compounds which can potentially help human prevent disease related to stress of oxidation (Seczyk, Swieca, Kapusta, & Gawlik-Dziki, 2019). Many epidemiological studies have indicated that countries with a high intake of pulses have lowered risks of some chronic diseases (Bessada et al., 2019). There is high concentration of bioactive substance, such as polyphenols, flavonols and tannins in cowpea seeds, which is associated with a wide range of beneficial health properties such as prevention of inflammatory, cardiovascular disease and type-2 diabetes (Adjei-Fremah et al., 2019) Due to its nutritional value, cowpea has been incorporated in a

variety of cereal-based food products, such as noodle, cookie, muffin, bread and extrusion breakfast (Adjei-Fremah et al., 2019).

Starch digestibility is another essential factor for starchy food because starch digestibility is associated with human health. Starch in human diets is digested into its simpler glucose units by several enzymes in the human body, including salivary α -amylase and pancreatic amylase (Whitney and Rolfes, 2007). The starch digestion rate and the human body blood sugar response can indicate the association between digestibility of starch and the health of human.

The glycemic index (GI) is used to classify different type of carbohydrates food consumed based on blood glucose after having meal (Jenkins et al., 1982). Many studies used the predictive glycaemic response to evaluate the response of blood glucose after consuming food (Brennan et al., 2012a). It is detrimental to human health and associated with some chronic diseases to consume food with a high glycemic index (GI) for the long term. Therefore, lowering the glycemic index of food is an excellent way to prevent chronic diseases (Jia et al., 2020). Generally, legumes have low glycemic index (GI) because their high-level dietary fibre content, starch chemical structure and physicochemical properties play an essential role in digestion. Properties of different starch also influence the starch digestibility. The proportion of amylopectin contained in starch may influence the digestibility of starch. Generally, starch containing high ratio of amylopectin shows higher starch digestibility than high amylose content starch. Legume starch contains higher proportion of amylose (30-40%) and lower percentage of amylopectin (60-70%) compared with most of other food starch which contains 25-30% amylose and 70-75% amylopectin (Singh, Dartois, & Kaur, 2010). Cowpea flours are considered as a good source of low glycemic index (GI) foods because cowpea flours contain high-level slowly digestible starch and dietary fibre (Tinus, Damour, Van Riel, & Sopade, 2012).

Generally, the in vitro glycaemic response was reduced by adding legume flours in a starchy food product (Monnet et al., 2019). However, the effect of incorporation of legume flour to cereal product on starch digestibility is influenced by the amount of legume flour added and the type of fibre in the legume flour. Gularte, Gómez, & Rosell (2012) incorporated legume flour to rice flour to make gluten-free food and found the addition of legume flour reduced GI of final products because the proportion of rapidly digestible starch dropped in the final product. Another study found low level (5%) addition of pea flour into pasta did not affect its starch digestibility, while 15% pea flour added into durum wheat spaghetti significantly reduced the starch digestibility, which is possibly caused by the changes in the pasta structure (Padalino et al., 2014). Also, a study found that high concentration fortification of bean flour in brown rice decreased the rapidly digestible starch and increased the resistant starch of the extrusion product due to the increase of total dietary fibre from bean flour

(Sumargo, Gulati, Weier, Clarke, & Rose, 2016). However, Tudorică et al. (2002) found that incorporating 7.5% insoluble fibre (pea fibre) into wheat pasta made the starch easier to digest by enzyme because the insoluble fibre broke the protein matrix surrounding the starch granules. Also, Brennan, Lan, & Brennan (2016) found that the incorporation of 10% pea flour to barley pasta did not significantly alter the starch digestibility while adding the same amount of oat reduced the starch digestibility significantly. The possible reason is that oats contain β -glucan fibre, which can increase the viscosity of starch and in turn, inhibit the starch hydrolysis.

Therefore, the effect of the addition of dietary fibre in legume to cereal product on starch digestibility is also related to the type of fiber. Incorporation of soluble dietary fiber may inhibit the starch digestion while a certain amount of insoluble fiber might have the opposite effect on starch digestibility. Tudorică, Kuri, & Brennan (2002) studied the effect of fortification of dietary fiber (pea fiber or guar gum) into pasta on starch digestibility. They indicated that soluble fiber added into cereal product contributed to the inhibition of starch digestion, but insoluble fiber increased the starch digestibility of pasta because the insoluble fiber may break the protein-starch matrix, leading to the increase of starch hydrolysis by the enzyme. By contrast, the soluble fiber (for example, guar gum)-protein-starch matrix might protect the starch granules from the hydrolysis by the enzyme. Jia et al. (2020) reported that incorporating soluble dietary fibre in biscuits influenced the starch digestibility and rheological attributes of the dough by altering the physical and chemical properties of the starch matrix. Another study found the incorporation of 25% chickpea flour to durum wheat pasta inhibited in vitro starch hydrolysis and decreased the in vivo glycaemic index, because pasta with chickpea flour incorporated contains high concentration of the oligosaccharides and indigestible fraction such as nonstarch polysaccharides and resistant starch (Goñi & Valentín-Gamazo, 2003).

Incorporation of protein to rice flour not only influences the nutrition of rice starch but also might affect starch digestibility and the pasting properties because of the synergistic of protein and starch in the formulation. Addition of protein in rice flour may alter the digestion rate of starch. Many previous studies have reported that the fortification of protein in rice starch or wheat starch influenced the digestibility of starch due to the interaction of protein and starch (Chen et al., 2017). A study indicated that even a small amount of protein incorporated could change starch digestibility (Cockcroft et al., 2012). A comprehensive review examined a wide range of factors influencing starch digestibility such as composition, processing, protein and lipid (Singh et al., 2010). An early study used pronase enzyme to digest the protein-starch matrix and significantly improved the starch digestibility because the enzyme can access starch granules. This finding demonstrated that the protective role of protein on starch granules (Rooney & Pflugfelder, 1986). Recent research examined the synergistic effect of incorporating exogenous protein to corn starch on starch digestibility and

rheological attributes and indicated the presence of protein retarded the gelatinisation of starch granules and inhibited the hydrolysis of starch (Yang, Zhong, Douglas Goff, & Li, 2019). Another recent study also explored the effect of pea protein and whey protein on pasting properties and starch digestibility of two types of rice starch (Oñate Narciso & Brennan, 2018). The study found the addition of protein reduced the pasting properties of rice starch paste due to the synergistic effect of protein and starch. Also, the study found that the increasing proportion of protein decreased the sugar released of glutinous rice starch which has low content of amylose during in vitro starch digestion.

Despite that there are many previous studies have examined the effect of incorporation of legume protein or whey protein or legume flour on the nutritional and physicochemical properties of wheat and rice starch individually, the synergistic impact of the fortification of both legume flour and whey protein on rice starch properties has not been studied widely.

1.1 Objective

The present study will research the effect of the fortification of both legume flour (cowpea flour) and whey protein on rice starch properties by examining antioxidant properties, pasting attributes and starch digestibility of the blended samples composed of different ratio of cowpea flour, whey protein and rice flour.

1.2 Hypotheses:

The antioxidant property of samples will increase with the incorporation of cowpea flour and whey protein in rice flour.

The starch digestibility of samples will decrease with the increasing proportion of cowpea flour and whey protein in the samples.

The pasting property of samples will decrease with the increasing proportion of cowpea flour and whey protein in the samples.

Chapter 2

Material and Methods

2.1 Materials

Table 2-1 Sample recipes

Sample name	Formulation
R	Raw Rice flour
C	Raw Cowpea flour
W	whey protein concentrate (WPC)
F1	R:C 90:10
F2	R:C:W 80:15:05
F3	R:C:W 70:20:10
F4	R:C:W 60:25:15
F5	R:C:W 50:30:20

2.2 Antioxidant property measurement

2.2.1 Preparation of sample extracts

Weigh out 2 g of sample powder and transfer to a 50 mL plastic sample pottle then add 20 mL of 70% methanol solution. Stirring sample for overnight using the multi-stirrer at 20 °C. put the samples in the centrifuge and set the speed at 2500 rpm and time for 10 minutes, after centrifuge, transfer the supernatant to plastic tubes and label them, then store at -20 °C for analysis.

2.2.2 Measurement of Total Phenolics Content (TPC)

The total phenolic content is measured according to the method (Giusti, Caprioli, Ricciutelli, Vittori, & Sagratini, 2017). First, prepare standard solutions, namely, gallic acid at 0, 12.5, 25, 50, 75, 100, and 150 µg/mL in 70% methanol. Second, take 0.5 mL of each standard and sample extract in different tubes, then add 2.5 mL of Folin-Ciocalteu reagent (0.2 N) and 2.0 mL of sodium carbonate (7.5%) to each tube and mix well. Prepare samples and standards in triplicates. Third, after incubation of in a water bath at 40 °C for 30 min, cool samples and standards to room temperature for at least 5 min. Use a spectrophotometer to measure the absorbance for each standard and sample at 760 nm. The results are presented as (gallic acid equivalents / gram weight).

2.2.3 Measurement of Radical Scavenging Capacity (ABTS)

First, prepare 10mL 7 mM ABTS stock solution in water. Second, take 0.27g of K₂S₂O₈ to make up to 10 mL with methanol in 10 mL volumetric flask in order to make 10 mL of 100 mM Potassium

Persulfate stock solution in water. Third, the day before assay, add 9.5 mL of 7mM ABTS stock solution and 245 μL of 100 mM $\text{K}_2\text{S}_2\text{O}_8$ (potassium persulfate) solution in a volumetric flask, then make up to 10 mL solution with water. Use foil to cover the solution to protect from light. Keep the solution in a dark place at room temperature overnight, allowing the reaction to stand for more than 16 hours. Fourth, on the day of analysis, use PBS (pH 7.4) dilute the ABTS radical reagent solution to an absorbance of 0.70 (± 0.02) at 734 nm. Prepare standard solutions 0 – 200 μmol Trolox in 70% methanol. Transfer 3 mL of diluted ABTS radical reagent solution to each cuvette and add 300 μL of each Trolox standard or sample extract and mix well. After incubation for 6 minutes at room temperature, use a spectrophotometer to measure the absorbance of each standard and sample at 734 nm. The assay is carried out in triplicate. The results are expressed as μmol of Trolox equivalents/ gram sample weight (Wang et al., 2016).

2.3 Viscosity Measurement using Rapid Visco Analyzer (RVA)

The measurement of pasting property uses AACC Method 76-21, 1999. The weight of ingredients of samples is shown in Table 2.2. Measure accurately 3.40 g of each sample flour (14% moisture basis) directly into each test canister. Measure 25.1 ± 0.1 ml distilled water and add it into each new test canister. Transfer the sample onto the surface of water in the canister. Use the stirrer mix sample rapidly and then measure the pasting property by a Rapid Viscosity Analyser (Perten Instruments, Hägersten, Sweden). The temperature and time profile are setting as following Table 2.2. Read the peak viscosity, breakdown and final viscosity values from the report. All samples are analysed in triplicates.

Table 2-2 The temperature and time profile for RVA analysis

Stage	Temperature/ Speed	STD1
1	50°C	0 min, 0 sec
2	960 rpm	0 min, 0 sec
3	160 rpm	0 min, 10 sec
4	50°C	1 min, 0 sec
5	95°C	4 min, 42 sec
6	95°C	7 min, 12 sec
7	50°C	11 min, 0 sec
End of test		13 min, 0 sec
Time between readings		4 sec

AACC Method 76-21, 1999

Table 2-3 Raw cowpea flour, rice flour, and five formulation samples for RVA analysis. The rice flour was added with cowpea flour and whey protein concentrate (WPC) as five different formulations (F1-F5).

Sample name	Formulation	Rice (g)	Cowpea (g)	WPC (g)	Total weight of sample (g)	Volume of Added Water (ml)
R	Raw Rice flour	3.40			3.40	25.10
C	Raw Cowpea flour		3.40		3.40	25.10
W	WPC			3.40	3.40	25.10
F1	R:C 90:10	3.06	0.34		3.40	25.10
F2	R:C:W 80:15:05	2.72	0.51	0.17	3.40	25.10
F3	R:C:W 70:20:10	2.38	0.68	0.34	3.40	25.10
F4	R:C:W 60:25:15	2.04	0.85	0.51	3.40	25.10
F5	R:C:W 50:30:20	1.70	1.02	0.68	3.40	25.10

2.4 In Vitro Starch Digestion

Use the following method (Oñate Narciso & Brennan, 2018) to measure the amount of glucose released of each sample during in vitro digestion for 120 min at 37 °C. In summary, weight 2.5g each sample in triplicate, then adds 0.8 mL of 1 M HCl to each sample container. Prepare a pepsin solution (10 %) in 0.05 M HCl when the temperature has reached 37 °C. Add 10% pepsin to each sample container and allow samples digest for 30 min at 37 °C. Add 2 mL 1 M NaHCO₃, 5 mL 0.1 M sodium maleate buffer (pH 6) to each sample container. Transfer 1 mL sample from each sample container to each new falcon tube with 4ml ethanol in the tube. Label these samples as “time 0” digestion aliquots. Add 0.1 mL of amyloglucosidase and 5 mL of the 2.5% pancreatin solution to each sample container, then start timing for digestion. Add 10 mL of RO water to each sample container to accurately make volume up to 53 mL for the digestion. Incubate for 120min at 37 °C, take 1 ml aliquot at 20min, 60min, 120min point to each new falcon tube with 4ml ethanol added. The enzyme used for gastric digestion includes pepsin (Acros Organics, New Jersey, USA CAS:901-75-6) and pancreatin (Applichem GmbH, Darmstadt, Germany CAS: 8049-47-6, activity: 42362 FIP-U/g).

2.5 Statistical analysis

All experiments were performed in triplicate. Data were analysed by one-way analysis of variance (ANOVA) using Minitab 18 software and Tukey's comparison test ($p > 0.05$).

Chapter 3

Results and Discussion

3.1 Antioxidant property

3.1.1 Total Phenolics Content (TPC)

Table 3-1 The content of total phenolics of cowpea flour, rice flour, and the five different ratio formulation samples.

Samples name	Formulations	Total phenolics content (mg GAE/g FW)	Std. dev
Rice four	Raw Rice flour	0.107 ^g	0.002
Cowpea flour	Raw Cowpea flour	0.888 ^a	0.007
WPC	Whey protein concentrate	0.691 ^b	0.022
F1	R:C 90:10	0.276 ^f	0.048
F2	R:C:W 80:15:05	0.308 ^{ef}	0.002
F3	R:C:W 70:20:10	0.354 ^{de}	0.007
F4	R:C:W 60:25:15	0.402 ^d	0.045
F5	R:C:W 50:30:20	0.490 ^c	0.005

Total phenolics content is expressed as mg gallic acid equivalents per gram fresh weight.

Data represent the mean values and standard deviation for each sample (n = 3)

Data are analysed by one-way ANOVA and Tukey's comparison test.

Means that do not share a letter are significantly different ($p \leq 0.05$).

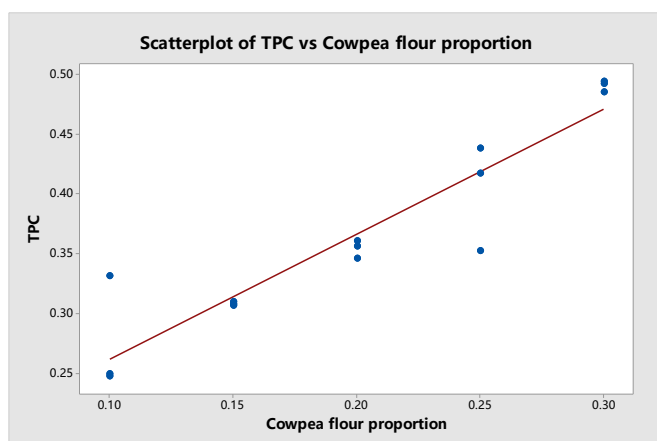


Figure 3-1 Regression analysis of the percentage of cowpea flour and TPC of samples ($P \leq 0.05$)

$R^2 = 87.37\%$

Regression Equation, $TPC = 0.1575 + 1.044 \text{ Cowpea flour proportion}$

3.1.2 Measurement of Radical Scavenging Capacity (ABTS assay)

Table 3-2 Results of Radical Scavenging Capacity (ABTS) of raw cowpea flour, rice flour, and five formulation samples.

Samples name	Formulations	Total phenolics ($\mu\text{mol Trolox/g}$ fresh weight)	Std. dev
Rice four	Raw Rice flour	3.090 ^b	2.321
Cowpea flour	Raw Cowpea flour	10.506 ^a	0.391
F1	R:C 90:10	2.947 ^b	0.157
F2	R:C:W 80:15:05	4.034 ^b	0.279
F3	R:C:W 70:20:10	4.921 ^b	0.189
F4	R:C:W 60:25:15	4.590 ^b	0.174
F5	R:C:W 50:30:20	5.060 ^b	0.157

Total phenolics content is expressed as $\mu\text{mol Trolox}$ per gram fresh weight (FW).

Data represent the mean values and standard deviation for each sample ($n = 3$)

Data are analysed by one-way ANOVA and Tukey's comparison test.

Means that do not share a letter are significantly different ($p \leq 0.05$).

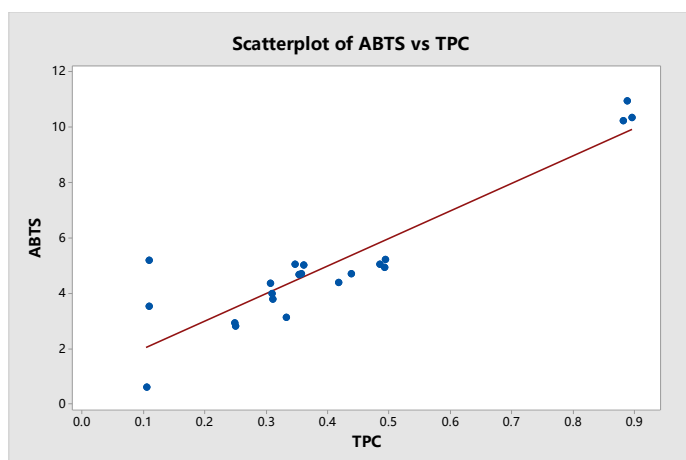


Figure 3-2 Regression analysis of ABTS Radical Scavenging Capacity and TPC of samples ($P \leq 0.05$)

$R^2 = 83.46\%$

Regression Equation **$\text{ABTS} = 0.990 + 9.99 \text{ TPC}$**

According to regression analysis, there is a significant positive correlation between mean TPC of the samples and the proportion of cowpea flour incorporated ($P < 0.05$). The content of total phenolics of cowpea flour, rice flour, and the five different ratio formulation samples are listed in Table 3-1 The content of total phenolics of cowpea flour, rice flour, and the five different ratio formulation samples.. Rice four has the lowest total phenolics content, 0.107 mg GAE/g FW, while cowpea flour

has the highest total phenolics content, about eight times TPC of Rice flour. Many studies have demonstrated that legume flour contains a high content of total phenolics. The TPC in legume flour is correlated with the dark colour coat of the legume. Pulses with dark colour seed coat have higher antioxidant than those with pale seed coat (Giusti et al., 2017). With the proportion of cowpea flour increasing in the samples, the TPC of samples increased gradually. From the Figure 3-1 Regression analysis of the percentage of cowpea flour and TPC of samples ($P \leq 0.05$), it can be seen mean TPC of the samples has a significant positive correlation with the proportion of cowpea flour ($P \leq 0.05$), with of 87.37% the variation in TPC explained by the proportion of cowpea flour. This is consistent with a previous study, which found that the increase of the ratio of carob bean flour incorporated to rice flour increased the total phenolics content and antioxidant activity of both mixture flour samples before extrusion and the extruded products (Arribas, Cabellos, Cuadrado, Guillamón, & Pedrosa, 2019).

Cowpea flour has the highest ABTS radical scavenging capacity, 3.090 $\mu\text{mol Trolox/g}$ fresh weight, about three times of that of rice flour. The potential radical scavenging capacity increased with the incorporation of cowpea flour to rice flour. However, there is no significant difference in ABTS value between samples (except cowpea flour) ($P > 0.05$). As expected, according to regression analysis ($R^2 = 83.46\%$), there is a significant positive correlation between total phenolic content with ABTS radical scavenging capacity ($p \leq 0.05$) in the present case.

The whey protein concentrate contains phenolic compounds. In this case, the TPC of WPC is $0.691 \pm 0.022 \text{ mg GAE/g FW}$. Besides cowpea flour, the TPC in WPC also contributes to the TPC of five different ratio samples, with the increasing percentage of WPC in the formulations. Also, protein and peptides have antioxidant abilities because particular AA can play a role as metal chelators and hydrogen donors. Peptides have a synergistic influence with phenolic compounds on antioxidant properties (Bessada et al., 2019) Further study on individual and synergistic effects of both cowpea flour and WPC on the TPC of the samples need to be done by incorporating cowpea flour and WPC individually into the formulations.

The phenolic compounds in pulse mainly include phenolic acids, tannins, flavonoids and anthocyanins. These compounds have antioxidant attributes which are beneficial to human body system against oxidation. However, some phenolic compounds, such as tannins, polyphenols, are antinutritional factors (ANF), which may reduce the digestibility of protein (Bessada et al., 2019). Therefore, the effect of phenolic compounds on the protein digestibility need to be further studied, when incorporating cowpea flour and whey protein together to the rice flour.

3.2 Viscosity Measurement by RVA

Table 3-3 Results of RVA analysis of raw cowpea flour, rice flour, and five formulation samples.

Samples	Peak1		breakdown		Final Viscosity	
	Average	Std. dev	Average	Std. dev	Average	Std. dev
Cowpea flour	1266.3 ^e	52.7	279.3 ^c	4.5	1553.3 ^g	96.7
Rice flour	4003.3 ^a	200.2	973.0 ^a	129.3	7098.7 ^a	142.3
F1 R:C 90:10	3593.0 ^b	143.4	559.7 ^b	42.8	6121.0 ^b	167.0
F2 R:C:W 80:15:05	2465.7 ^c	23.5	187.0 ^{cd}	2.6	4976.0 ^c	49.4
F3 R:C:W 70:20:10	1707.5 ^d	33.2	30.0 ^d	4.2	3596.5 ^d	81.3
F4 R:C:W 60:25:15	1235.7 ^e	73.4	30.7 ^d	9.9	2617.3 ^e	108.0
F5 R:C:W 50:30:20	1044.5 ^e	12.0	47.5 ^d	3.5	1946.5 ^f	26.2

The rice flour was added with cowpea flour and whey protein concentrate (WPC) as five different ratio formulation samples (F1-F5).

Data represent the mean values and standard deviation for each sample (n=3, except F3, F5 were analysed in duplicates)

Data are analysed by one-way ANOVA and Tukey's comparison test.

Means that do not share a letter are significantly different ($p \leq 0.05$).

According to the results of RVA, the addition of cowpea flour and whey protein has a significant effect on the pasting properties of the blend starch. The cowpea flour has a much lower peak viscosity and break down viscosity and final viscosity compared with that of rice flour. Overall, the peak, breakdown and final viscosity of samples gradually decreased with the increasing ratio of cowpea flour and whey protein concentrate. However, according to ANOVA analysis and Tukey comparison test of RVA results, the peak viscosity of F1 to F3 and cowpea flour, rice flour is significantly different ($P \leq 0.05$) while there is no significant difference between F4, F5 samples and cowpea flour in peak viscosity ($P > 0.05$). This means the peak viscosity increased significantly by incorporating cowpea flour and whey protein at a low level ($P \leq 0.05$). While, when the ratio of cowpea flour and whey protein increased to 40%, the influence on peak viscosity became not significant. Similarly, the breakdown values also did not significantly differ among F2-F5 samples, which means low concentration (10% cowpea flour) made a significant difference on breakdown viscosity ($P \leq 0.05$), while the effect of higher-level incorporation of cowpea flour and whey protein was not significant ($P > 0.05$). Interestingly, the final viscosity differed significantly among all samples ($P \leq 0.05$).

Three main reasons possibly contribute to the decline of the peak and final viscosity in the present work. First, the proportion of amylose and amylopectin in a starch influences the starch pasting property through affecting gelatinisation and retrogradation of starch during cooling stage (Varavinit,

Shobsngob, Varayanond, Chinachoti, & Naivikul, 2003). With the proportion of cowpea flour increasing, rice starch ratio decreases, leading to a decrease of amylopectin and an increase of amylose in the starch samples because cowpea flour contains more amylose than rice flour. The amylopectin has a positive impact on pasting properties of starch-protein gel while amylose does not have (Yang et al., 2019).

Another possible reason is the synergistic effect of protein- starch composite on viscosity property. A recent study examined the effect of the incorporation of pea protein and whey protein in rice flour on the pasting properties of blend flour (Oñate Narciso & Brennan, 2018). They indicated that the increasing proportion of whey protein concentrates or pea protein isolates significantly decreased the peak, break down and final viscosity of basmati starch. The interaction between starch and protein is influenced by the composition of the starch, the proportion of the amylopectin and amylose. Lentil starch has low amylopectin but high content of amylose while rice starch contains less amylose (about 15-20%) and more amylopectin (about 80-85%) on a weight basis (Benmoussa, Moldenhauer, & Hamaker, 2007). The cowpea flour is rich in protein compared with rice flour. The cowpea protein in the formulation increased with the increasing proportion of the cowpea flour. When incorporating cowpea flour and whey protein to take place part of rice flour, the proportion of rice starch decreased, and the percentage of cowpea starch increased. Therefore, the synergetic effect of starch and protein on the pasting attributes of their composite gel decreased with the decrease of amylopectin content. The reduction of final viscosity may be caused by that the amylose chain is not able to retrograde during the stage of cooling because of the presence of proteins (Yang et al., 2019).

The value of breakdown shows how fragile the granular of starch breaks down after the viscosity of the starch gel reaches the peak point. The breakdown viscosity of samples significantly dropped when incorporating cowpea flour and whey protein as formulation 1 and 2. However, formulation 3, 4,5 showed a different pattern, and their break down viscosity are similar and maintained at a low level. This finding agrees with another previous study (Joshi, Aldred, Panozzo, Kasapis, & Adhikari, 2014), which researched the rheology attributes of the different ratio of lentil starch and lentil protein combination gel. The study concluded that starch dominant lentil starch-protein composite gel showed the typical viscosity property as starch. Break down viscosity increased with the increase of starch ratio. By contrast, with the increasing proportion of protein, the breakdown viscosity decreased sharply, and almost lost break down viscosity when the protein became a significant part of the formulation. Another factor that affects breakdown viscosity is the ratio of rapidly digestible starch (RDS) and slowly digestible starch (SDS) in the formulations. The RDS has a positive correlation with breakdown viscosity, while SDS has a negative correlation with breakdown viscosity (Chung, Liu,

Lee, & Wei, 2011). Cowpea flour contains high content slowly digestible starch than rice starch (Adjei-Fremah et al., 2019), therefore decrease the breakdown viscosity.

The third possible reason is the dietary fiber and resistant starch contained in cowpea flour. A study has demonstrated that fortification of dietary fiber (Inulin and soluble oat fiber) and resistant starch in wheat flour decreased the viscosity values of the mixture flour (Blanco Canalis, León, & Ribotta, 2019). They indicated that the starch granules for gelatinisation decreased when incorporating dietary fiber. Also, the peak viscosity is related to the water binding. The dietary fiber absorbed more water than wheat flour and thereby affect the starch granules to swell. This may explain why the peak viscosity decreased with the increasing addition of cowpea flour. The breakdown values are related to the resistance of the paste and lower breakdown means more resistance of the paste. Incorporation of dietary fiber hinders the starch granules from swelling while increasing the resistance of the paste (Blanco Canalis et al., 2019). This can explain why the incorporation of cowpea flour to rice flour decreases the breakdown value in the present experiment.

In the present case, we did not add a higher percentage (>30%) of cowpea flour in the rice starch. By contrast, another study examined the effect of high percentages of brown cowpea flour (25%, 50%, 75%) incorporated in rice flour (without whey protein) on the pasting properties of the blended flour (Iwe, Onyeukwu, & Agiriga, 2016). They found that 25% and 50% addition of cowpea flour significantly decreased the peak viscosity and break down viscosity of the blended flour. However, 75% incorporation of cowpea flour significantly increased the peak viscosity and breakdown viscosity compared with that of 50% cowpea flour content mixture. They indicated that high starch content is the reason to explain this result. However, 50% cowpea flour: 50% rice flour blend has more starch than the blend contains 75% cowpea flour and 25% rice flour because cowpea flour contains more protein and dietary fiber and less starch than rice flour. So, there may be other causes. A previous study indicated that slowly digestible starch content has a negative correlation with breakdown viscosity of the starch and the RVA breakdown viscosity can be used to predict starch digestibility (Benmoussa et al., 2007). Also, Liu et al. (2018) reported the slowly digestible starch content in extrusion product that made from rice grain and soybean dietary fiber increased, with the increasing ratio of soybean fiber, however, it began to decrease when the addition of soybean fiber reached 6%. The study indicated that dietary fiber incorporated might embed the rapidly digestible starch granules and thus reduce the proportion of the rapidly digestible starch, thereby hinder the starch digestion. Therefore, the slowly digestible starch may increase with the increasing proportion of cowpea flour at low level, but it might decrease at high concentration of cowpea flour. Therefore, the effect of cowpea flour added in rice flour on the pasting property and starch digestibility needs to be further studied at higher proportion level without whey protein.

3.3 In Vitro Starch Digestion

The glucose released (mg glucose/g sample) after in vitro starch digestion of cowpea flour, rice flour, and five different formulations of cowpea flour, rice flour and whey protein at 0, 20, 60, 120 min point is shown in the figure.

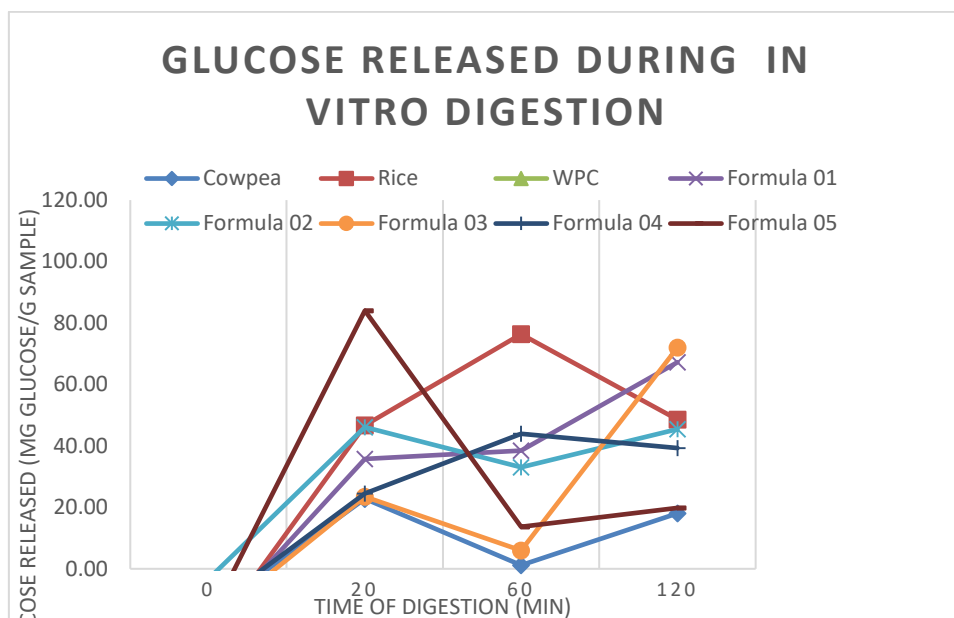


Figure 3-3 The glucose released (mg glucose/g sample) during in vitro starch digestion of cowpea flour, rice flour, and five different formulations of cowpea flour, rice flour and whey protein at 0, 20, 60, 120 mins.

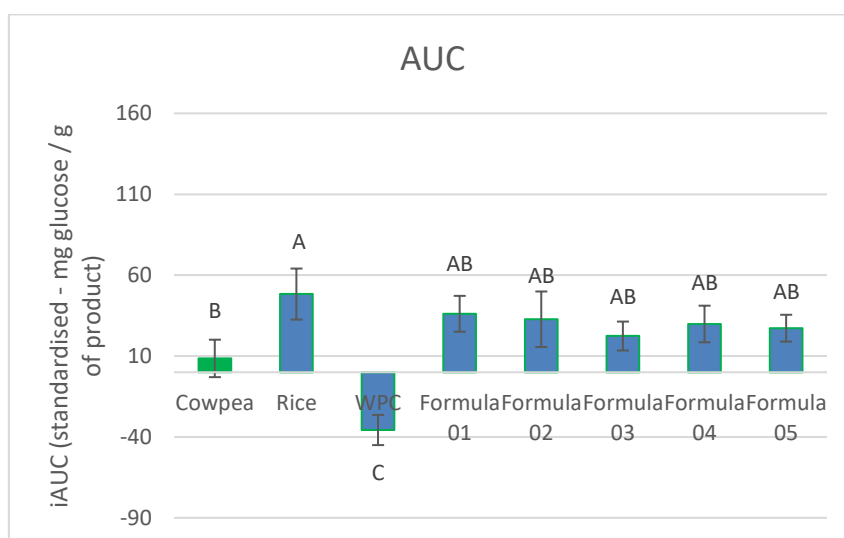


Figure 3-4 Values for area under the curve (AUC) of five different formulations of cowpea flour, rice flour and whey protein.

Data are analysed by one-way ANOVA and Tukey's comparison test.

Means that do not share a letter are significantly different ($p \leq 0.05$).

According to the results shown in the above Figure 3-3 The glucose released (mg glucose/g sample) during in vitro starch digestion of cowpea flour, rice flour, and five different formulations of cowpea flour, rice flour and whey protein at 0, 20, 60, 120 mins., Figure 3-4 Values for area under the curve (AUC) of five different formulations of cowpea flour, rice flour and whey protein., the amount of glucose released of rice flour, cowpea flour and whey protein are significantly different ($p \leq 0.05$). The incorporation of whey protein and cowpea flour altered the starch digestibility of samples, although there is no significant difference in the amount of glucose released of five formulations ($P > 0.05$). Overall, the amount of glucose released of the samples decreased during in vitro starch digestion with the increased proportion of whey protein and cowpea flour in the formulations, except the formulation 3 sample. The finding is in line with a previous study that the incorporation of protein (either Whey protein concentrate or Pea protein isolate) had a significant effect on digestibility of glutinous starch (low content of amylose) (Oñate Narciso & Brennan, 2018). The study found the amylose content in starch influenced the starch digestibility and glutinous starch digestibility of the mixture decreased with the ration of whey protein or pea protein isolate increased. The study also indicated that the reduction of starch portion and the inhibition of hydrolysis of starch by protein might cause a decrease in sugar realised during starch digestibility. Another similar study examined the effect of the interaction of whey protein and corn starch on starch digestibility (Yang et al., 2019). They found the incorporation of whey protein isolate significantly increase the amount of slowly digestible starch and resistant starch and decrease the rapidly digestible starch. They indicated that the whey protein played a role as a physical barrier surrounded the granules of starch and thereby retarding the hydrolysis of starch. In the present work, incorporation of WPC reduced the starch in the samples. Addition of cowpea flour in the rice flour, not only reduced the rice starch but also increase cowpea starch and cowpea protein. Cowpea flour is high in slowly digestive starch, protein and dietary fibre (Adjei-Fremah et al., 2019). Therefore, the amount of sugar-reduced because of the decrease in starch and increasing ratio of slowly digestible starch. Another mechanism is the fortification of protein (both WPC and cowpea protein) may inhibit the hydrolysis of starch by binding on the surface of starch and prevent granules of starch from hydrolysis (Chen et al., 2017).

The addition of legume flour into cereal also may affect the starch digestibility. A study reported the incorporation of legume flour in cereal decreased the rapidly digestible starch, inhibited the starch hydrolysis rate and reduced the starch digestibility, thereby lowering the estimated glycemic index of the starch (Gularte et al., 2012). Another study found an increasing proportion of bean flour in brown rice decreased the rapidly digestible starch and increased the resistant digestible starch of the

extrusion product because of the increase of total dietary fiber from bean flour (Sumargo et al., 2016).

Dietary fibre in cowpea flour possibly contributes to the reduction of the sugar released during in vitro starch digestion, but the effect is associated with the type, and the amount of the dietary fiber added. A previous study examined the effect of adding soluble dietary fibre in biscuits on the glycemic index. The study demonstrated that the glucose released during in vitro starch digestion reduced with the addition of soluble dietary fibre (Jia et al., 2020). The possible mechanism is that soluble dietary fibre can influence the physical and chemical attributes of the starch. Soluble dietary fibre can increase the viscosity of the starch gel matrix. Therefore, soluble dietary fibre retards the starch digestion from the digestive effect of enzymes by encapsulating starch grains, leading to a reduction of glucose released (Juvonen et al., 2009). However, another study found the incorporation of pea fiber (mainly insoluble fiber) into pasta did not inhibit the hydrolysis of pasta starch while guar was able to decrease the starch digestibility of pasta significantly. They indicated that the effect is associated with the type of dietary fiber.

Soluble fiber can reduce starch digestibility. However, insoluble fiber may increase the starch digestibility when added at low concentration because insoluble fiber may damage the pasta matrix and starch-protein matrix, leading to more opportunities for the enzyme to access the starch (Tudorică et al., 2002). Another similar study reported the fortification of one-fourth chickpea flour to durum wheat-based pasta inhibited in vitro starch hydrolysis and decrease the in vivo glycaemic index of the pasta because pasta fortified with chickpea flour is high in the oligosaccharides and indigestible fraction such as nonstarch polysaccharides and resistant starch (Goñi & Valentín-Gamazo, 2003). Therefore, If a high proportion of legume flour is incorporated into cereal, the starch digestibility may decrease because of the presence of indigestible fraction. Cowpea flour contains about 12.00 to 14.80 g per 100 g total dietary fiber. After cooking, the proportion of insoluble fiber in cowpea is around three times that of soluble fiber (Jayathilake et al., 2018). Therefore, in this case, the overall starch digestion is inhibited by addition of whey protein and legume flour, but the effect of dietary fiber and indigestion fraction in cowpea flour on the starch digestibility needs to be further studied.

Chapter 4

Conclusion

This study examined the effect of incorporating cowpea flour and whey protein to rice flour on the antioxidant property, pasting property and the starch digestibility of the samples.

There is a significant positive correlation between mean TPC of the samples and the proportion of cowpea flour incorporated ($P \leq 0.05$). Also, there is a significant positive correlation between total phenolic content with ABTS radical scavenging capacity ($P \leq 0.05$). As expected, cowpea flour has the highest total phenolic content, and rice flour has the lowest. The effect of phenolic compounds on the protein digestibility and the influence of protein on phenolic compounds need to be further studied when incorporating cowpea flour and whey protein together to the rice flour.

According to the analysis of RVA results, the addition of cowpea flour and whey protein has a significant effect on the pasting properties of the blended flour. The peak viscosity, break down viscosity and final viscosity of the cowpea flour are much lower than that of rice flour. The peak, breakdown and final viscosity of samples decreased gradually with the increasing proportion of cowpea flour and whey protein concentrate. However, according to ANOVA analysis and Tukey comparison test of RVA results, the peak viscosity of F1 to F3 and cowpea flour, rice flour is significantly different ($P \leq 0.05$) while there is no significant difference between F4, F5 samples and cowpea flour in peak viscosity ($P > 0.05$). This means the peak viscosity increased significantly by the incorporation of cowpea flour and whey protein at a low level, while the influence on peak viscosity became not significant at high-level addition. Similarly, the breakdown values also did not significantly differ among F2-F5 samples, which means low concentration of cowpea flour 10% has a significant effect on breakdown viscosity ($P \leq 0.05$), while the effect of higher-level incorporation was not significant ($P > 0.05$). The final viscosity differed significantly among all samples ($P \leq 0.05$).

This finding is possibly caused by three main reasons in the present study. First, the proportion of amylose and amylopectin in starch may influence the starch gel pasting property through affecting gelatinisation and retrogradation of starch during the cooling stage. With the increase of the proportion of cowpea flour incorporated in the samples, the ratio of amylose increased while amylopectin content decreased. The synergetic effect of starch and protein on the pasting attributes of the blended flour is another reason. The reduction of final viscosity may be caused by that the amylose chain is not able to retrograde during the stage of cooling due to the presence of proteins. The breakdown viscosity of samples also significantly dropped when incorporating cowpea flour and whey protein. Cowpea flour contains high content slowly digestible starch than rice starch, and the

rapidly digestible starch has a positive correlation with breakdown viscosity, while slowly digestible starch has a negative correlation with breakdown viscosity, thereby decreasing the breakdown viscosity. Also, with the increasing ratio of cowpea dietary fiber, the rapidly digestible starch content may decrease because that dietary fiber might embed the rapidly digestible starch granules and thus reduce the proportion of the rapidly digestible starch, thereby hinder the starch digestion. The third reason is that the dietary fiber contained in cowpea flour absorbed more water than rice flour and thereby affect the starch granules to swell, thereby decreasing peak viscosity. The breakdown values are related to the resistance of the paste and lower breakdown means more resistance of the paste. Incorporation of dietary fiber hinders the starch granules from swelling, while increasing the resistance of the paste, thereby decreasing the breakdown values.

Based on the in vitro starch digestion analysis, the incorporation of whey protein and cowpea flour affected the starch digestibility of samples. Overall, the amount of reducing sugar released of the samples decreased during in vitro starch digestion with the increase portion of whey protein and cowpea flour in the formulations due to the decrease in starch and increasing ratio of slowly digestive starch from cowpea flour. Another reason is that protein can inhibit the starch hydrolysis by binding on the starch surface and hinder granules of starch from hydrolysis. Also, the dietary fiber and indigestible fraction in cowpea flour play an essential role in the starch digestibility. Dietary fibre (soluble and insoluble) in cowpea flour possibly contributes to the reduction of the sugar released during in vitro starch digestion, but the effect is associated with the type and the amount of the dietary fiber. Cowpea flour contains mainly insoluble fiber. Soluble fiber has been demonstrated that it decrease the starch digestibility. However, insoluble fiber may increase the starch digestibility when incorporated at a low level because insoluble fiber may break the starch-protein matrix, and thus the enzyme is easier to access starch. The effect of cowpea flour added in rice flour on the pasting property and starch digestibility needs to be further studied using higher proportions of cowpea flour.

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